REMARKS

The specification has been amended to include sequence identification numbers inadvertingly omitted at the time of filing. This amendment is submitted to place the patent application in accordance with the sequence rules 37 C.F.R. 1.821 - 1.825 Applicants respectfully request examination on the merits of this application.

The title of the application has been amended to conform with the Initial Information Data Sheet and Transmittal that were filed with the subject application on August 27, 2001. In addition, the U.S. Patent number of a referenced application, previously identified by serial number and filing date, is provided for clarity.

Attached hereto is a marked-up version of the changes made to the Specification by the current amendment. It is entitled: "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 300622002611. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The title has been amended as follows:

[Polyketide Synthase Enzymes and Recombinant DNA Constructs Therefor]

Polynucleotides Encoding the *fkbA* Gene of the FK-520 Polyketide Synthase Gene Cluster

The paragraph beginning at page 26, line 17 has been amended as follows:

Referring to Figures 1 and 3, the FK-520 PKS gene cluster is composed of four open reading frames designated *fkbB*, *fkbC*, *fkbA*, and *fkbP*. The *fkbB* open reading frame encodes the loading module and the first four extender modules of the PKS. The *fkbC* open reading frame encodes extender modules five and six of the PKS. The *fkbA* open reading frame encodes extender modules seven, eight, nine, and ten of the PKS. The *fkbP* open reading frame encodes the NRPS of the PKS. Each of these genes can be isolated from the cosmids of the invention described above. The DNA sequences of these genes are provided below (SEQ ID NO:1) preceded by the following table identifying the start and stop codons of the open reading frames of each gene and the modules and domains contained therein.

Nucleotides	Gene or Domain
complement (412 - 1836)	*fkbW*
complement (2020 - 3579)	*fkbV*
complement (3969 - 4496)	*fkbR2*
complement (4595 - 5488)	*fkbR1*
5601 - 6818	*fkbE*
6808 - 8052	*fkbF*
8156 - 8824	*fkbG*
complement (9122 - 9883)	*fkbH*
complement (9894 - 10994)	*fkbI*
complement (10987 - 11247)	*fkbJ*
complement (11244 - 12092)	*fkbK*
complement (12113 - 13150)	*fkbL*
complement (13212 - 23988)	*fkbC*
complement (23992 - 46573)	*fkbB*
46754 - 47788	*fkbO*
47785 - 52272	*fkbP*
52275 - 71465	*fkbA* (SEQ ID NO:72)

71462 - 72628	*fkbD*
72625 - 73407	*fkbM*
complement (73460 - 76202)	*fkbN*
complement (76336 - 77080)	*fkbQ*
complement (77076 - 77535)	*fkbS*
complement (44974 - 46573)	CoA ligase of loading domain
complement (43777 - 44629)	ER of loading domain
complement (43144 - 43660)	ACP of loading domain
complement (41842 - 43093)	KS of extender module 1 (KS1)
complement(40609 - 41842)	AT1
complement (39442 - 40609)	DH1
complement (38677 - 39307)	KR1
complement (38371 - 38581)	ACP1
complement (37145 - 38296)	KS2
complement (35749 - 37144)	AT2
complement (34606 - 35749)	DH2 (inactive)
complement (33823 - 34480)	KR2
complement (33505 - 33715)	ACP2
complement (32185 - 33439)	KS3
complement (31018 - 32185)	AT3
complement (29869 - 31018)	DH3 (inactive)
complement (29092 - 29740)	KR3
complement (28750 - 28960)	ACP3
complement (27430 - 28684)	KS4
complement (26146 - 27430)	AT4
complement (24997 - 26146)	DH4 (inactive)
complement (24163 - 24373)	ACP4
complement (22653 - 23892)	KS5
complement (21420 - 22653)	AT5
complement (20241 - 21420)	DH5
complement (19464 - 20097)	KR5
complement (19116 - 19326)	ACP5
complement (17820 - 19053)	KS6
complement (16587 - 17820)	AT6
complement (15438 - 16587)	DH6
complement (14517 - 15294)	ER6
complement (13761 - 14394)	KR6
complement (13452 - 13662)	ACP6
52362 - 53576	KS7
53577 - 54716	AT7
54717 - 55871	DH7
56019 - 56819	ER7
56943 - 57575	KR7
5771[0]1 - 57920	ACP7
57990 - 59243	KS8
59244 - 60398	AT8

60399 - 61412	DH8 (inactive)
61548 - 62180	KR8
62328 - 62537	ACP8
62598 - 63854	KS9
63855 - 65084	AT9
65085 - 66254	DH9
66399 - 67175	ER9
67299 - 67931	KR9
68094 - 68303	ACP9
68397 - 69653	KS10
69654 - 70985	AT10
71064 - 71273	ACP10

The paragraph beginning on page 77, line 3 has been amended as follows:

To construct a hybrid PKS or FK-520 derivative PKS gene of the invention, one can employ a technique, described in PCT Pub. No. 98/27203 and U.S. patent application Serial No. 08/989,332, filed 11 Dec. 1997, now U.S. Patent No. 6,033,883, each of which is incorporated herein by reference, in which the large PKS gene is divided into two or more, typically three, segments, and each segment is placed on a separate expression vector. In this manner, each of the segments of the gene can be altered, and various altered segments can be combined in a single host cell to provide a recombinant PKS gene of the invention. This technique makes more efficient the construction of large libraries of recombinant PKS genes, vectors for expressing those genes, and host cells comprising those vectors.

The paragraph beginning on page 80, line 8 has been amended as follows:

For 2-hydroxymalonyl CoA biosynthesis, the *fkbH*, *fkbI*, *fkbJ*, and *fkbK* genes are sufficient to confer this ability on *Streptomcyces* host cells. For conversion of 2-hydroxymalonyl to 2-methoxymalonyl, the *fkbG* gene is also employed. While the complete coding sequence for *fkbH* is provided on the cosmids of the invention, the sequence for this gene provided herein may be missing a T residue, based on a comparison made with a similar gene cloned from the

ansamitocin gene cluster by Dr. H. Floss. Where the sequence herein shows one T, there may be two, resulting in an extension of the *fkbH* reading frame to encode the amino acid sequence (SEQ ID NO:2):

The paragraph beginning on page 86, line 25 has been amended as follows:

To construct an expression cassette for performing module 8 AT domain replacements in the FK-520 PKS, a 4.6 kb *Sph*I fragment from the FK-520 gene cluster was cloned into plasmid pLitmus 38 (a cloning vector available from New England Biolabs). The 4.6 kb *Sph*I fragment, which encodes the ACP domain of module 7 followed by module 8 through the KR domain, was isolated from an agarose gel after digesting the cosmid pKOS65-C31 with *Sph* I. The clone having the insert oriented so the single *Sac*I site was nearest to the *Spe*I end of the polylinker was identified and designated as plasmid pKOS60-21-67. To generate appropriate cloning sites, two linkers were ligated sequentially as follows. First, a linker was ligated between the *Spe*I and *Sac*I sites to introduce a *Bgl*II site at the 5' end of the cassette, to eliminate interfering polylinker sites, and to reduce the total insert size to 4.5 kb (the limit of the phage KC515). The ligation reactions contained 5 picomolar unphosphorylated linker DNA and 0.1 picomolar vector DNA, i.e., a 50-fold molar excess of linker to vector. The linker had the following sequence (SEQ ID NOS:3-4):

5'-CTAGTGGGCAGATCTGGCAGCT-3'
3'-ACCCGTCTAGACCG-5'

The resulting plasmid was designated pKOS60-27-1.

The paragraph beginning on page 87, line 12 has been amended as follows:

Next, a linker of the following sequence was ligated between the unique *Sph*I and *Afl*II sites of plasmid pKOS60-27-1 to introduce an *Nsi*I site at the 3' end of the module 8 cassette. The linker employed was (SEQ ID NOS:5-6):

5'-GGGATGCATGGC-3'
3'-GTACCCCTACGTACCGAATT-5'

The resulting plasmid was designated pKOS60-29-55.

The paragraph beginning on page 87, line 18 has been amended as follows:

To allow in-frame insertions of alternative AT domains, sites were engineered at the 5' end (*Avr* II or *Nhe* I) and 3' end (*Xho* I) of the AT domain using the polymerase chain reaction (PCR) as follows. Plasmid pKOS60-29-55 was used as a template for the PCR and sequence 5' to the AT domain was amplified with the primers SpeBgl-fwd and either Avr-rev or Nhe-rev (SEQ ID NOS:7-9):

SpeBgl-fwd 5'-CGACTCACTAGTGGGCAGATCTGG-3'

Avr-rev 5'-CACGCCTAGGCCGGTCGGTCTCGGGCCAC-3'

Nhe-rev 5'-GCGGCTAGCTGCTCGCCCATCGCGGGATGC-3'

The paragraph beginning on page 88, line 6 has been amended as follows:

Plasmid pKOS60-29-55 was again used as a template for PCR to amplify sequence 3' to the AT domain using the primers BsrXho-fwd and NsiAfl-rev (SEQ ID NOS:10-11):

BsrXho-fwd 5'-GATGTACAGCTCGAGTCGGCACGCCCGGCCGCATC-3'

NsiAfl-rev 5'-CGACTCACTTAAGCCATGCATCC-3'

The paragraph beginning on page 88, line 16 has been amended as follows:

Malonyl and methylmalonyl-specific AT domains were cloned from the rapamycin cluster using PCR amplification with a pair of primers that introduce an *Avr*II or *Nhe*I site at the 5' end and an *Xho*I site at the 3' end. The PCR conditions were as given above and the primer sequences were as follows (SEQ ID NOS:12-15):

RATN1 5'-ATCCTAGGCGGGCRGGYGTGTCGTCCTTCGG-3'

(3' end of Rap KS sequence and universal for malonyl and methylmalonyl CoA),

RATMN2 5'-ATGCTAGCCGCCGCGTTCCCCGTCTTCGCGCG-3'

(Rap AT shorter version 5'- sequence and specific for malonyl CoA),

RATMMN2 5'-ATGCTAGCGGATTCGTCGGTGGTGTTCGCCGA-3'

(Rap AT shorter version 5'- sequence and specific for methylmalonyl CoA), and

RATC 5'-ATCTCGAGCCAGTASCGCTGGTGYTGGAAGG-3'

(Rap DH 5'- sequence and universal for malonyl and methylmalonyl CoA).

The paragraph beginning on page 89, line 16 has been amended as follows:

The *Avr*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 12 of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below. The AT of rap module 12 is specific for incorporation of malonyl units (SEQ ID NOS:16-17).

The paragraph beginning on page 92, line 49 has been amended as follows:

The *Avr*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 13 (specific for methylmalonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:18-19).

The paragraph beginning on page 96, line 28 has been amended as follows:

The *Nhe*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 12 (specific for malonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:20-21).

The paragraph beginning on page 100, line 1 has been amended as follows:

The *Nhe*II-*Xho*I restriction fragment that encodes module 8 of the FK-520 PKS with the endogenous AT domain replaced by the AT domain of module 13 (specific for methylmalonyl CoA) of the rapamycin PKS has the DNA sequence and encodes the amino acid sequence shown below (SEQ ID NOS:22-23).

The paragraph beginning on page 105, line 24 has been amended as follows:

The naturally occurring module 8 sequence for the MA6548 strain is shown below, followed by the illustrative hybrid module 8 sequences for the MA6548 strains (SEQ ID NOS:24-25).

The paragraph beginning on page 109, line 21 has been amended as follows:

The *Avr*II-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 12 of rapamycin is shown below (SEQ ID NOS:26-27).

The paragraph beginning on page 112, line 51 has been amended as follows:

The *Avr*II-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 13 of rapamycin is shown below (SEQ ID NOS:28-29).

The paragraph beginning on page 116, line 32 has been amended as follows:

The *Nhe*I-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 12 of rapamycin is shown below (SEQ ID NOS:30-31).

The paragraph beginning on page 120, line 11 has been amended as follows:

The *Nhe*I-*Xho*I hybrid FK-506 PKS module 8 containing the AT domain of module 13 of rapamycin is shown below (SEQ ID NOS:32-33).

The paragraph beginning on page 125, line 1 has been amended as follows:

The following table shows the location and sequences surrounding the engineered site of each of the heterologous AT domains employed (SEQ ID NOS:34-63, in order of appearance). The FK-506 hybrid construct was used as a control for the FK-520 recombinant cells produced, and a similar FK-520 hybrid construct was used as a control for the FK-506 recombinant cells.

The paragraph beginning on page 126, line 1 has been amended as follows:

The sequences shown below provide the location of the KS/AT boundaries chosen in the FK-520 module 8 coding sequences. Regions where *Avr*II and *Nhe*I sites were engineered are indicated by lower case and underlining (SEQ ID NOS:64-65).

The paragraph beginning on page 126, line 21 has been amended as follows:

The sequences shown below provide the location of the AT/DH boundary chosen in the FK-520 module 8 coding sequences. The region where an *Xho*I site was engineered is indicated by lower case and underlining (SEQ ID NOS:66-67).

The paragraph beginning on page 126, line 29 has been amended as follows:

The sequences shown below provide the location of the KS/AT boundaries chosen in the FK-506 module 8 coding sequences. Regions where *Avr*II and *Nhe*I sites were engineered are indicated by lower case and underlining (SEQ ID NOS:68-69).

The paragraph beginning on page 127, line 4 has been amended as follows:

The sequences shown below provide the location of the AT/DH boundary chosen in the FK-506 module 8 coding sequences. The region where an *Xho*I site was engineered is indicated by lower case and underlining (SEQ ID NOS:70-71).